During the reactions the solutions turned from plum colored to green. The solvent was removed by evaporation under reduced pressure, and the crude products were purified by preparative thin-layer chromatography using precoated silica gel plates (20 × 20 cm and 2-mm thickness) as adsorbent and $CHCl_3$–MeOH (9:1, v/v) as the developing solvent. The products were recrystallized from CH_2Cl_2–MeOH or other solvents specified in Table I. This table also gives the yields and properties of the products.

Acknowledgment. This investigation was supported by Grant CA 21430, awarded by the National Cancer Institute, DHHS, and by funds from Bristol Laboratories.

Registry No. 1, 4049-15-4; 2, 86689-59-0; 3, 86689-60-3; 4, 4154-16-9; 5, 86689-61-4; 6, 84397-50-2; 7, 86689-62-5; 8, 86689-63-6; 9, 17287-49-9; 10, 84397-25-1; 11, 17269-53-3; 12, 84397-44-4; 13, 86689-64-7; 14, 86689-65-8; 15, 4349-74-0; 16, 84397-42-2; 17, 86689-66-9; 18, 84397-43-3; 19, 84397-26-2; 20, 4117-86-6; 21, 86709-35-5; 22, 86689-67-0; 23, 84397-24-0; 24, 86689-68-1; 25, 86689-69-2; 26, 86689-70-5; $(C_2H_5)_2NH$, 109-89-7; $(CH_3)_2NCH_2CH_2NHCH_3$, 142-25-6; c-$O(CH_2CH_2)_2NH$, 110-91-8; c-$S(CH_2CH_2)_2NH$, 123-90-0; c-$HN(CH_2CH_2)_2NCH_3$, 109-01-3; c-$HN(CH_2CH_2)_2NCHO$, 7755-92-2; mitomycin A, 4055-39-4; 3-methylpiperidine, 626-56-2; 3-hydroxypiperidine, 6859-99-0; 4,4-dihydroxypiperidine, 73390-11-1; 4-piperidylpiperidine, 4897-50-1; *N*-(4-acetylphenyl)piperazine, 51639-48-6; 3-pyrroline, 109-96-6; 3-hydroxypyrrolidine, 40499-83-0; thiazolidine, 504-78-9; indoline, 496-15-1; 2-phenylaziridine, 1499-00-9; 2-cyanoaziridine, 33898-53-2; 2-carboxamidoaziridine, 5950-35-6; 2-methoxycarbonylaziridine, 5950-34-5; 2-ethoxycarbonylaziridine, 5950-36-7.

Supplementary Material Available: Full screening data for compounds submitted to the P-388 assay (Table II) (5 pages). Ordering information is given on any current masthead page.

Inhibition of Renin by Angiotensinogen Peptide Fragments Containing the Hydroxy Amino Acid Residue 5-Amino-3-hydroxy-7-methyloctanoic Acid

Rodney L. Johnson* and Karen Verschoor

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
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The 3*R*,5*S* and 3*S*,5*S* diastereoisomers of the hydroxy amino acid 5-amino-3-hydroxy-7-methyloctanoic acid (AHMOA) were synthesized from L-leucine and then incorporated into various peptide fragments of angiotensinogen to give the following polypeptides: AHMOA-Val-Phe-OCH_3, His-AHMOA-Val-Phe-OCH_3, and AHMOA-Ile-His-OCH_3. These compounds were tested in an in vitro renin assay system for their ability to inhibit either hog kidney renin or human amniotic renin. The most active analogue of the series was (3*R*,5*S*)-AHMOA-Val-Phe-OCH_3 (**16**). Against hog kidney renin, this compound possessed a $K_i = 1.7 \times 10^{-4}$ M, while against human amniotic fluid, **16** had a $K_i = 0.95 \times 10^{-4}$ M. The analogues AHMOA-Val-Phe-OCH_3 and His-AHMOA-Val-Phe-OCH_3 exhibited noncompetitive kinetics when the 3*R*,5*S* isomer of AHMOA was employed and competitive kinetics when the 3*S*,5*S* diastereoisomer of AHMOA was used.

Because of the important role that the renin–angiotensin system plays in the regulation of blood pressure and blood volume in both physiological and pathophysiological states, there has been an ongoing effort by many investigators to develop antagonists of the renin–angiotensin system as either pharmacological tools or as therapeutic agents.[1-3] Inhibition of renin has been thought to be one means by which the renin–angiotensin system could be blocked, since the reaction between renin and its substrate, angiotensinogen, is the rate-limiting step in the sequence of enzymatic reactions that make up the renin–angiotensin system.

We have been attempting to develop new types of renin inhibitors by utilizing an approach that involves the design and synthesis of compounds that might mimic the postulated transition state of the renin–angiotensinogen reaction. Although renin's mechanism of catalysis remains unknown, two models have been proposed as a result of studies that have been conducted on renin and related aspartyl proteases.[4-7] In one model, it is postulated that the carboxyl group of one of the aspartic acid residues known to be present within the active site of renin attacks the Leu[10] amide carbonyl group. A general-base mechanism of hydrolysis is postulated for model 2. In this model, the carbonyl carbon of the scissile peptide bond is attacked by a molecule of water. The result in both cases is the formation of a tetrahedral intermediate (Figure 1) wherein the Leu[10] carbonyl group is transformed into a hydroxyl group and the amide nitrogen atom of residue 11 begins to take on the characteristics of an amino nitrogen.

In previous studies aimed at mimicking the postulated tetrahedral intermediates shown in Figure 1, we modified the known substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3[8] in two ways. In one modification the N-terminal leucyl residue of Leu-Leu-Val-Phe-OCH_3 was replaced with various α-hydroxyalkanoyl residues.[9] This modification yielded inhibitors of renin that were up to five times more active than Leu-Leu-Val-Phe-OCH_3. A second modification consisted of substituting the hydroxy amino acid residue 3-amino-2-hydroxy-5-methylhexanoic acid for the leucyl residues of Leu-Leu-Val-Phe-OCH_3.[10] This modification yielded inhibitors that exhibited competitive or noncompetitive kinetics, depending on the

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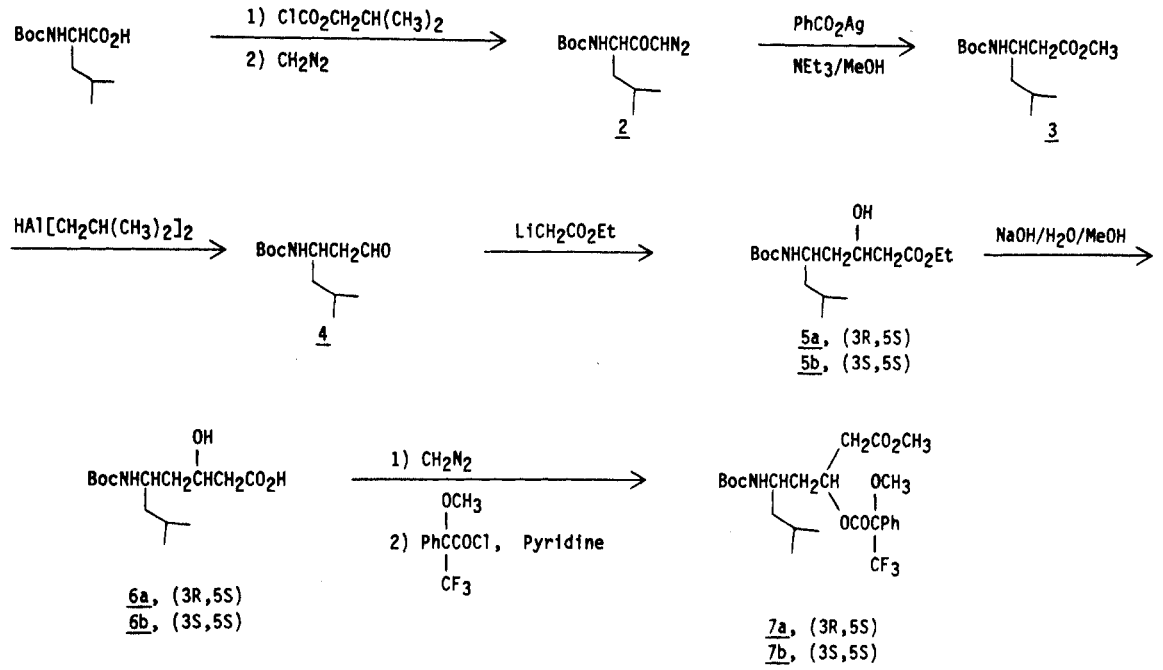
0022-2623/83/1826-1457$01.50/0

Scheme I



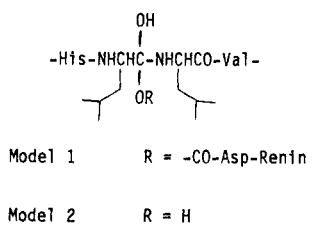
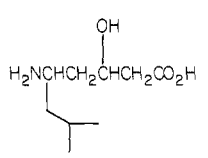


Figure 1. Hypothetical tetrahedral intermediates formed during renin's reaction with its substrate angiotensinogen.

stereochemistry of the hydroxy amino acid residue.

In the present study, we report the synthesis of the hydroxy amino acid 5-amino-3-hydroxy-7-methyloctanoic acid (1, AHMOA) and the renin inhibitory activity of an-



1 (AHMOA)

giotensinogen peptide fragments in which this hydroxy amino acid has been incorporated in order to mimic the postulated tetrahedral intermediate formed during the reaction between renin and angiotensinogen.

Results and Discussion

Chemistry. The *tert*-butoxycarbonyl derivative of the hydroxy amino acid 5-amino-3-hydroxy-7-methyloctanoic acid (1) was synthesized as outlined in Scheme I. The starting material for this synthesis was Boc-L-Leu. The reaction of this material with isobutyl chloroformate yielded a mixed anhydride, which on subsequent treatment with diazomethane afforded the diazo ketone **2** in an 80% yield. Treatment of **2** with silver benzoate by the method of Stachowiak et al.[11] gave Boc-L-β-Hle methyl ester **3** in an 89% yield. The ester was reduced to aldehyde **4** with diisobutylaluminum hydride, and this material, in turn, was reacted with lithium ethyl acetate using reaction conditions similar to those described by Rich and coworkers,[12] to give ethyl 5-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-7-methyloctanoate (**5**) as a mixture of two diastereoisomers, **5a** and **5b**. These two diastereoisomers were readily separated from one another by a low-pressure silica gel column. Hydrolysis of **5a** and **5b** with NaOH in aqueous methanol yielded the respective free acids **6a** and **6b** in excellent yields. Compounds **6a** and **6b** were subsequently used in the synthesis of the polypeptides containing the diastereoisomeric 5-amino-3-hydroxy-7-methyloctanoic acid residues.

The stereochemical assignment of the C-3 carbon atom of the diastereoisomeric β-hydroxy esters **5a** and **5b** was made by using two indirect, but independent, methods. In one method the specific rotations obtained for the two diastereoisomers of **5** were compared with the specific rotations of analogous β-hydroxy esters. The β-hydroxy esters used for comparison were ethyl (3*S*,4*S*)- and (3*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-6-methylheptanoate[12] and ethyl (3*S*,4*S*)- and (3*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-phenylpentanoate.[13] In both of the above cases the diastereoisomer with the 3*S*,4*S* stereochemistry gave a more negative specific rotation in methanol than the 3*R*,4*S* diastereoisomer. Since the diastereoisomer of **5** that came off the silica gel column last, compound **5b**, gave a more negative specific rotation in methanol than the diastereoisomer that came off the silica gel column first, compound **5a**, the diastereoisomers **5a** and **5b** were assigned the 3*R*,5*S* and 3*S*,5*S* stereochemistry, respectively.

A similar comparison was also made between the specific rotations of the diastereoisomeric β-hydroxy acids **6a** and **6b** and the analogous β-hydroxy acids (3*S*,4*S*)- and (3*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-6-methylheptanoic acid[12] and (3*S*,4*S*)- and (3*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-phenylpentanoic acid.[13] As in the case of the β-hydroxy esters, the diastereoisomers with the 3*S*,4*S* stereochemistry gave

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Table I. Physical Properties of the Protected Polypeptides Containing (3*R*,5*S*)-AHMOA and (3*S*,5*S*)-AHMOA

no.	compound[a]	chirality[b]	yield, %	TLC, R_f	mp, °C	$[\alpha]_D^{25}$, deg (c, CH_3OH)	formula[c]
8	Boc-AHMOA-Val-Phe-OCH_3	3*R*,5*S*	76	0.36[d]	175-178	−28 (1.0)	$C_{29}H_{47}N_3O_7$
9	Boc-AHMOA-Val-Phe-OCH_3	3*S*,5*S*	69	0.33[d]	146-148	−30.6 (1.05)	$C_{29}H_{47}N_3O_7$
10	N^{π}-Bom-N^{α}-Boc-His-AHMOA-Val-Phe-OCH_3	3*R*,5*S*	79	0.58[e]	75-80[f]	−25.5 (1.1)	$C_{43}H_{62}N_6O_9$
11	N^{π}-Bom-N^{α}-Boc-His-AHMOA-Val-Phe-OCH_3	3*S*,5*S*	77	0.53[e]	145-148	−24 (1.0)	$C_{43}H_{62}N_6O_9$[g]
12	Boc-His-AHMOA-Val-Phe-OCH_3	3*R*,5*S*	98	0.57[h]	80-83[f]	−23.4 (1.0)	$C_{35}H_{54}N_6O_8$
13	Boc-His-AHMOA-Val-Phe-OCH_3	3*S*,5*S*	71	0.53[h]	141-144	−25.5 (1.0)	$C_{35}H_{54}N_6O_8$
14	Boc-AHMOA-Ile-His-OCH_3	3*R*,5*S*	60	0.57[h]	70-72	−13.8 (1.0)	$C_{27}H_{47}N_5O_7$
15	Boc-AHMOA-Ile-His-OCH_3	3*S*,5*S*	58	0.57[h]	116-119	−18.8 (1.0)	$C_{27}H_{47}N_5O_7$

[a] AHMOA refers to 5-amino-3-hydroxy-7-methyloctanoic acid. [b] Chirality of AHMOA residue. [c] All compounds were analyzed for C, H, and N and were within ±0.4% of the calculated values unless otherwise indicated. [d] $CHCl_3$-EtOAc (1:1), UV, and I_2 visualization. [e] $CHCl_3$-CH_3OH (9:1), UV, and I_2 visualization. [f] Obtained as a foam, which sinters at the indicated temperature. [g] C: calcd, 64.00; found, 62.59. [h] $CHCl_3$-CH_3OH (9:1), Pauly reagent visualization.

Table II. Physical Properties of the Deprotected Polypeptides Containing (3*R*,5*S*)-AHMOA and (3*S*,5*S*)-AHMOA

no.	compound[a]	chirality[b]	yield, %	TLC, R_f	mp, °C	$[\alpha]_D^{25}$, deg (c, CH_3OH)	formula[c]
16	AHMOA-Val-Phe-OCH_3·HCl	3*R*,5*S*	100	0.23[d]	95-98	−14.8 (1.0)	$C_{24}H_{40}N_3O_5Cl$
17	AHMOA-Val-Phe-OCH_3·HCl	3*S*,5*S*	90	0.30[d]	125-130	−26.5 (1.08)	$C_{24}H_{40}N_3O_5Cl$
18	His-AHMOA-Val-Phe-OCH_3·2HCl	3*R*,5*S*	73	0.76[e]	150[f]	−10 (1.05)	$C_{30}H_{48}N_6O_6Cl\cdot{}^1/_2H_2O$
19	His-AHMOA-Val-Phe-OCH_3·2HCl	3*S*,5*S*	83	0.72[e]	140[f]	−9 (1.0)	$C_{30}H_{48}N_6O_6Cl\cdot H_2O$
20	AHMOA-Ile-His-OCH_3·2HCl	3*R*,5*S*	65	0.64[e]	225-227	+7.3 (0.52)	$C_{22}H_{41}N_5O_5Cl_2$
21	AHMOA-Ile-His-OCH_3·2HCl	3*S*,5*S*	86	0.76[e]	184-187	−7.7 (1.0)	$C_{22}H_{41}N_5O_5Cl_2$[g]

[a] AHMOA refers to 5-amino-3-hydroxy-7-methyloctanoic acid. [b] Chirality of AHMOA residue. [c] All compounds were analyzed for C, H, and N and were within 0.4% of the calculated values unless otherwise indicated. [d] $CHCl_3$-CH_3OH (9:1) and ninhydrin visualization. [e] 1-Propanol-NH_4OH (4:1), ninhydrin, and Pauly reagent visualization. [f] Material sinters. [g] C: calcd, 50.18; found, 49.55.

Table III. Renin Inhibitory Activity of Angiotensinogen Peptide Fragments Containing the 5-Amino-3-hydroxy-7-methyloctanoic Acid Residue

no.	compound[a]	chirality[b]	K_i, 10^{-4} M (type of inhibn[c]) hog kidney	human amniotic
16	AHMOA-Val-Phe-OCH_3·HCl	3*R*,5*S*	1.7 (NC)	0.95 (NC)
17	AHMOA-Val-Phe-OCH_3·HCl	3*S*,5*S*	11.8 (C)	16 (C)
18	His-AHMOA-Val-Phe-OCH_3·2HCl	3*R*,5*S*	9 (NC)	30 (NC)
19	His-AHMOA-Val-Phe-OCH_3·2HCl	3*S*,5*S*	10 (C)	7.4 (C)
22	His-Leu-Leu-Val-Phe-OCH_3·2HCl		0.8 (C)	5.4 (C)

[a] AHMOA refers to 5-amino-3-hydroxy-7-methyloctanoic acid (1). [b] Chirality of AHMOA. [c] C = competitive; NC = noncompetitive.

a greater negative rotation in methanol than did the 3*R*,4*S* diastereoisomers. Isomer **6b** gave a more negative rotation than did isomer **6a** and, thus, was assigned the 3*S*,5*S* stereochemistry. This assignment was consistent with the stereochemical assignment given the precursor of **6b**, compound **5b**.

The second method that was used to assign the absolute configuration of the C-3 carbon atom of **5a** and **5b** employed the method developed by Yasuhara and Yamaguchi.[14] In this method, **6a** and **6b** were converted to the (*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetyl (MTPA) derivatives **7a** and **7b**, respectively, by the method of Dale and Mosher.[15] NMR spectra of **7a** and **7b** were taken in the presence and absence of 1 equiv of Eu(fod)$_3$, and the lanthanide-induced shift of the peak due to the $COOCH_3$ group (LIS_{COOMe}) was measured. The LIS_{COOMe} for **7a** was found to be 1.82 ppm, while the LIS_{COOMe} for **7b** was found to be 2.44 ppm. Based on the experiments of Yasuhara and Yamaguchi,[13] which showed that the LIS_{COOMe} for the (*R*)-MTPA derivatives of 3-(*S*)-hydroxy carboxylate esters is larger than the LIS_{COOMe} for the (*R*)-MTPA derivatives of 3(*R*)-hydroxy carboxylate esters, the chirality of C-3 carbon atom of **7b** was assigned the *S* configuration and that of **7a** was assigned the *R* configuration, since the LIS_{COOMe} for **7b** was larger than that observed in **7a**. Since **7a** and **7b** are derived from **5a** and **5b**, respectively, this means that the stereochemistry of **5a** is 3*R*,5*S* and that of **5b** is 3*S*,5*S*, a finding that agrees with the conclusions of the method which utilized a comparison of specific rotations.

The *tert*-butoxycarbonyl derivatives **6a** and **6b** were coupled to Val-Phe-OCH_3 and Ile-His-OCH_3 by using the dicyclohexylcarbodiimide/1-hydroxybenzotriazole method of coupling[16] to give the respective protected polypeptides **8**, **9**, **14**, and **15** (Table I). Treatment of these Boc-protected polypeptides with 4 N HCl in dioxane yielded the desired angiotensinogen peptide fragment analogues **16**, **17**, **20**, and **21**, respectively (Table II).

Polypeptides **16** and **17** were used in the synthesis of analogues **18** and **19**. Polypeptides **16** and **17** were coupled to N^{α}-(*tert*-butoxycarbonyl)-N^{π}-[(benzyloxy)methyl]-L-histidine[17] by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give the protected polypeptides **10** and **11**. The (benzyloxy)methyl (Bom) group was removed from **10** and **11** by hydrogenolysis over 10% Pd/C to give **12** and **13**, respectively. Removal of the *tert*-butoxycarbonyl group from **12** and **13** afforded the desired

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analogues **18** and **19**, respectively.

Renin Inhibition Studies. Compounds **16–22** were tested for their ability to inhibit both hog kidney renin and human amniotic renin[18] by using an in vitro renin assay system previously described by us.[9,10] Each compound's inhibitory activity and its inhibitory constant (K_i) were determined with Dixon plots.[19] The results are summarized in Table III.

Polypeptide **16** was found to be the most active renin inhibitor among the angiotensinogen peptide fragments that were synthesized and tested in this study. Compound **16** was found to have an inhibitory constant of 1.7×10^{-4} M against hog kidney renin. This tetrapeptide analogue was even a better inhibitor of human amniotic renin, where an inhibitory constant of 0.95×10^{-4} M was observed. Previously, we had reported that against human amniotic renin the tetrapeptide Leu-Leu-Val-Phe-OCH_3 possessed a K_i equal to 11.4×10^{-4} M.[9] Likewise, we have found that Leu-Gly-Val-Phe-OCH_3, a tetrapeptide that is even more closely related structurally to **16**, possesses an inhibitory constant equal to 10×10^{-4} M. From the present study it can be seen that replacing the Leu-Leu and Leu-Gly dipeptide segments of Leu-Leu-Val-Phe-OCH_3 and Leu-Gly-Val-Phe-OCH_3, respectively, with the hydroxy amino acid residue (3*R*,5*S*)-**1** affords an inhibitor of renin that is approximately 12 times more potent than either Leu-Leu-Val-Phe-OCH_3 or Leu-Gly-Val-Phe-OCH_3. When the diastereoisomeric hydroxy amino acid (3*S*,5*S*)-**1** replaces the Leu-Leu dipeptide segment of Leu-Leu-Val-Phe-OCH_3, we obtain a compound, analogue **17**, that is no better than Leu-Leu-Val-Phe-OCH_3 in inhibiting renin.

It was felt that the renin inhibitory activity of **16** and **17** could be increased if these peptide fragments were lengthened at the N-terminal end to include the amino acid residue that occurs next in the amino acid sequence of angiotensinogen. Thus, the histidine residue was added to **16** and **17** to yield analogues **18** and **19**, respectively. When the histidine residue was added to the tetrapeptide Leu-Leu-Val-Phe-OCH_3, the renin inhibitory activity of the resulting pentapeptide **22** was nearly doubled. Slightly better renin inhibitory activity against both hog kidney renin and human amniotic renin was also observed when analogue **17** was lengthened through the addition of an N-terminal histidine residue to give the pentapeptide analogue **19**. This was not the case when **16** was converted to the pentapeptide analogue **18**. Compound **18** was a much weaker inhibitor of renin than **16**. This was particularly true in the case of human amniotic renin, where it can be seen that **18** is some 30 times less potent in inhibiting this form of renin than is **16**. The reason for this difference probably lies in the different manner in which **16–19**, **22**, and Leu-Leu-Val-Phe-OCH_3 interact with renin. Compounds **17**, **19**, **22**, and Leu-Leu-Val-Phe-OCH_3 all probably bind to renin in a similar fashion, since they are all competitive inhibitors of renin, whereas compounds **16** and **18** probably bind to renin in a different manner, since these two analogues are noncompetitive inhibitors of renin. Thus, whereas the addition of a histidine residue to **17** and Leu-Leu-Val-Phe-OCH_3 appears to facilitate binding to the enzyme, this same change to **16** has an adverse affect on the ability of the resulting analogue to bind to renin.

Polypeptides **20** and **21** were synthesized in order to take into account the recent findings of Tewksbury et al.[20] who have shown that the amino terminal amino acid sequence of human angiotensinogen differs from hog angiotensinogen in that instead of having the Leu-Val-Tyr amino acid sequence at positions 11–13 it has the Val-Ile-His amino acid sequence. It was felt that **20** and **21** would show increased inhibitory activity against human amniotic renin. This did not, however, prove to be the case, since these two derivatives were found to be quite a bit less active than **16–19**. At a concentration of 10 mM, **20** inhibited hog kidney renin and human amniotic renin by 62 and 65%, respectively. The diastereoisomeric analogue **21**, at a concentration of 10 mM, was even less active, inhibiting hog kidney renin and human amniotic renin by only 40 and 33%, respectively. In light of the very weak activity shown by **20** and **21** and the fact that only limited amounts of these analogues were available, the extensive studies required to determine the inhibitory constant and mode of inhibition of the analogues were not carried out.

Interestingly, differences in the type of inhibition exhibited by polypeptides **16–19** were observed. These differences were found to be dependent upon the chirality of the AHMOA residue. The peptides that contained the (3*R*,5*S*)-AHMOA residue, compounds **16** and **18**, were found to be noncompetitive inhibitors of renin, while those peptides that contained the (3*S*,5*S*)-AHMOA residue, compounds **17** and **19**, were found to be competitive inhibitors of renin. We have observed this type of phenomenon before in our previous study[10] on the substitution of the N-terminal leucyl residues of Leu-Leu-Val-Phe-OCH_3 and Leu-Val-Phe-OCH_3 with the hydroxy amino acid 3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA). In this previous study, we found that with the analogues AHMHA-Leu-Val-Phe-OCH_3 and AHMHA-Val-Phe-OCH_3, competitive inhibition was observed when the AHMHA residue possessed the 2*S*,3*S* configuration, while noncompetitive inhibition was observed when the AHMHA residue possessed the 2*R*,3*S* stereochemistry. Thus, the results of this study and our previous study have demonstrated that the chirality of the carbon atom containing the hydroxyl moiety in the AHMOA and AHMHA residues has a significant effect on the way in which the peptide fragments containing these hydroxy amino acid residues interact with renin. When the carbon atom containing the hydroxyl moiety has the *R* configuration, noncompetitive inhibition of renin is observed, while when the configuration is *S*, competitive inhibition is seen.

Although it was hoped that substantial increases in renin inhibitory activity would be observed when a dipeptide mimic of the tetrahedral intermediate of the renin–antiotensinogen reaction was incorporated into angiotensinogen peptide fragments, this objective was not fully realized in the present study, where the 5-amino-3-hydroxy-7-methyloctanoic acid (AHMOA) residue was used for the dipeptide mimic of the tetrahedral intermediate of the renin–angiotensinogen reaction. Only modest increases in renin inhibitory activity were observed for the angiotensinogen peptide fragments that had the AHMOA residue incorporated within their structure. Whether this was because **1** was not a good mimic of the tetrahedral intermediate of the renin–angiotensinogen reaction or because the binding of the small peptide fragments containing the AHMOA residue to renin was such that the AHMOA residue was not in the proper position for it to be able to resemble the postulated tetrahedral intermediate

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remains to be determined through further study.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values. NMR specta were recorded on either a JOEL FX 90-MHz or a Brucker 250-MHz spectrometer. Low-pressure chromatography (20–40 psi) was carried out on Silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF uniplates. Visualization was done with UV and I_2. Hog kidney renin (lot no. 41F-8550) and porcine angiotensinogen (lot no. 102F-8584) were obtained from the Sigma Chemical Co., St. Louis, MO.

3(*S*)-[(*tert*-Butoxycarbonyl)amino]-1-diazo-5-methyl-2-hexanone (2). Boc-L-Leu (19.9 g, 0.086 mol) and *N*-methylmorpholine (8.7 g, 0.086 mol) were dissolved in THF (200 mL) and cooled to −30 °C in a dry ice/acetone cold bath. Isobutyl chloroformate (11.7 g, 0.086 mol) dissolved in THF (50 mL) was then added dropwise while maintaining the reaction temperature between −20 and −30 °C. Cold diethyl ether (200 mL) at −78 °C was added immediately, and the reaction mixture filtered while keeping the filter flask cold with a dry ice/acetone cold bath. The filtrate was added dropwise to an ethereal solution of diazomethane cooled to −20 °C. The reaction was stirred at 0 °C for 1 h and then at room temperature overnight. The solvent was removed under reduced pressure to give a yellow oil, which was taken up in ether and washed with distilled water, 1 M $NaHCO_3$ solution, and saturated NaCl solution. The organic phase was dried (Na_2SO_4) and then concentrated under reduced pressure to yield a yellow oil, which crystallized upon standing at room temperature. The crystals were triturated with cold petroleum ether (bp 30–60 °C) and filtered, yielding 17.6 g (80%) of light yellow crystals: mp 89.5–90 °C; $[\alpha]^{20}_D$ −78.3° (*c* 2.51, MeOH); NMR ($CDCl_3$) δ 5.44 (s, 1 H, CHN_2), 4.8–5.0 (br d, 1 H, NH), 3.92–4.2 (m, 1 H, NCHCO), 1.15–1.9 [m, with singlet at δ 1.42, 12 H, CH_2CH and $(CH_3)_3$], 0.97 [d, 6 H, *J* = 6 Hz, $(CH_3)_2$]. Anal. ($C_{12}H_{21}N_3O_3$) C,H,N.

Methyl 3(*S*)-[(*tert*-Butoxycarbonyl)amino]-5-methylhexanoate (3). To a solution of 3(*S*)-[(*tert*-butoxycarbonyl)amino]-1-diazo-5-methyl-2-hexanone (15.9 g, 62.3 mmol) in methanol (250 mL) was added, in a dropwise manner, a solution of silver benzoate (1.9 g, 8.3 mmol) in triethylamine (184 mL). The reaction was stirred overnight at room temperature, after which time it was treated with decolorizing charcoal. The solvent was removed from the filtrate under reduced pressure, and the brown residue that remained was taken up in ethyl acetate and treated with decolorizing charcoal. The filtrate was washed with saturated NaCl solution, dried over $MgSO_4$, and then stripped of solvent in vacuo to give a dark gold oil. This material was chromatographed on a low-pressure silica gel column using 20% ethyl acetate in methylene chloride as the eluting solvent to give 14.4 g (89%) of the purified ester as a light yellow oil: TLC (20% EtOAc/CH_2Cl_2) R_f 0.78; NMR ($CDCl_3$) δ 5.25 (br d, 1 H, amide NH), 3.9–4.2 (m, 1 H, NCH), 3.65 (s, 3 H, OCH_3), 2.72 (d, 2 H, CH_2CO), 1.15–1.95 [m, with singlet at δ 1.43, 12 H, CH_2CH and $(CH_3)_3$], 0.98 [d, 6 H, $(CH_3)_2$].

3(*S*)-[(*tert*-Butoxycarbonyl)amino]-5-methylhexanal (4). Methyl 3(*S*)-[(*tert*-butoxycarbonyl)amino]-5-methylhexanoate (4.6 g, 17.7 mmol) was dissolved in toluene (80 mL). The system was repeatedly flushed with N_2, and the solution was stirred at −78 °C under a stream of N_2. Diisobutylaluminum hydride in a hexane solution (37.2 mL, 37.2 mmol) was added dropwise over a 5-min period while maintaining the reaction temperature between −50 and −60 °C. After all the diisobutylaluminum hydride solution had been added to the reaction mixture, the reaction was immediately quenched by the cautious addition of methanol (4.5 mL), followed by the addition of a solution of sodium potassium tartrate (11.5 g) in distilled water (50 mL). The reaction mixture was allowed to warm to room temperature, where it was stirred for 2 h to break up the resultant colloidal suspension. Water and ether were added to the mixture, and the layers were separated. The aqueous layer was washed twice with ether, and the combined organic layers were washed with saturated NaCl solution and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a colorless oil, which crystallized upon standing. Purified aldehyde (3.5 g, 85%) was recovered by eluting the crude reaction product from a low-pressure silica gel column using 20% EtOAc/CH_2Cl_2 (R_f 0.67). A portion of the isolated aldehyde was recrystallized from petroleum ether (bp 60–70 °C) to give fluffy white needles: mp 75.0–75.5 °C; $[\alpha]^{20}_D$ −51.7° (*c* 2.6; $CHCl_3$); NMR ($CDCl_3$) δ 9.72 (s, 1 H, CHO), 4.30–4.60 (m, 1 H, amide NH), 3.90–4.2 (m, 1 H, NCH), 2.56 (dd, 2 H, *J* = 6 and 2 Hz, CH_2CO), 1.15–1.95 [m, with singlet at δ 1.42, 12 H, CH_2CH and $(CH_3)_3$], 0.97 [d, 6 H, *J* = 6 Hz $(CH_3)_2$]. Anal. ($C_{12}H_{23}NO_3$) C, H, N.

Ethyl 5(*S*)-[(*tert*-Butoxycarbonyl)amino]-3(*RS*)-hydroxy-7-methyloctanoate (5). Dry, freshly distilled THF (10 mL) was cooled to −20 °C with a dry ice/CCl_4 cold bath and repeatedly flushed with N_2. Under a stream of N_2, diisopropylamine (4.28 mL, 30 mmol) was added via syringe, followed by *n*-butyllithium in hexane (19 mL, 30 mmol). The reaction mixture was stirred for 1 h under N_2 at −20 °C and then cooled to −78 °C. Dry, distilled ethyl acetate (3.0 mL, 30 mmol) was added via syringe. The reaction was stirred for 15 min at −78 °C, and then 3(*S*)-[(*tert*-butoxycarbonyl)amino]-5-methylhexanal (3.6 g, 14.7 mmol) dissolved in THF (20 mL) was added dropwise. The reaction was stirred for 15 min and then quenched with 2 mL of cold 10% HCl. The mixture was allowed to warm to room temperature, and the aqueous layer was subsequently acidified to pH 2 with 10% HCl and washed three times with ethyl acetate. The combined organic layers were washed with saturated NaCl solution and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield 3.8 g of **5** as a yellow oil. The crude diastereoisomeric mixture was placed on a low-pressure silica gel column and eluted with a gradient consisting of 10–50% EtOAc in methylene chloride. The two isomeric products were recovered as white crystalline solids after recrystallization from petroleum ether (bp 30–60 °C).

Ethyl 5(*S*)-[(*tert*-butoxycarbonyl)amino]-3(*R*)-hydroxy-7-methyloctanoate (**5a**) was isolated in a yield of 1.2 g (48%): mp 78.5–79 °C; $[\alpha]^{20}_D$ 2.45° (*c* 2.44, MeOH), −8.69° (*c* 2.21, $CHCl_3$); TLC (20% EtOAc/CH_2Cl_2) R_f 0.56; NMR ($CDCl_3$) δ 4.43 (br d, 1 H, *J* = 8.8 Hz, NH), 4.09–4.20 (m, with quartet at δ 4.15, *J* = 7.3 Hz, 3 H, NHC*H*, OCH_2CH_3), 3.86–3.90 (m, 1 H, C*H*OH), 2.55 (dd, 1 H, *J* = 15 and 8 Hz, CH_2CO_2Et), 2.38 (dd, 1 H, *J* = 15 and 5.1 Hz, CH_2COOEt), 1.49–1.74 (m, 3 H, CH, CH_2), 1.45 [s, 9 H, $(CH_3)_3$], 1.30–1.42 (m, 2 H, CH_2), 1.26 (t, 3 H, *J* = 7.3 Hz, OCH_2CH_3), 0.91 [dd, 6 H, *J* = 6 and 1 Hz, $(CH_3)_2$]. Anal. ($C_{16}H_{31}NO_5$) C, H, N.

Ethyl 5(*S*)-[(*tert*-butoxycarbonyl)amino]-3(*S*)-hydroxy-7-methyloctanoate (**5b**) was isolated in a yield of 1.5 g (68%): mp 92.5–93 °C; $[\alpha]^{20}_D$ −10.15 (*c* 2.38, MeOH); $[\alpha]^{20}_D$ +4.69° (*c* 1.79, $CHCl_3$); TLC (20% EtOAc/CH_2Cl_2) R_f 0.41. NMR ($CDCl_3$) δ 4.42 (br d, 1 H, *J* = 9.1 Hz, NH), 4.08–4.21 (m, with quartet at δ 4.15, *J* = 7 Hz, 3 H, NHC*H*, OCH_2CH_3), 3.70–3.73 (m, 1 H, C*H*OH), 2.63 (dd, 1 H, *J* = 16.5 and 3.6 Hz, CH_2CO_2Et), 2.45 (dd, 1 H, *J* = 16.5 and 8.6 Hz, CH_2COOEt), 1.50–1.69 (m, 4 H, C*H*CH_2, CH_2CHOH), 1.43 [s, 9 H, $(CH_3)_3$], 1.24–1.36 (m, with triplet at δ 1.24, *J* = 7 Hz, 5 H, $CHCH_2$, OCH_2CH_3), 0.91 [d, 6 H, *J* = 6.6 Hz $(CH_3)_2$]. Anal. ($C_{16}H_{31}NO_5$) C, H, N.

5(*S*)-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-hydroxy-7-methyloctanoic Acid (6a). Hydroxy amino acid ester **5a** (1 g, 3.15 mmol) was dissolved in MeOH (15 mL). The solution was cooled in an ice bath and then treated with 2 mL of 10% NaOH (5 mmol). The solution was stirred at 0 °C for 10 min and then at room temperature for 1 h. At this time the methanol was removed in vacuo, and the resulting solution was washed with EtOAc. The aqueous layer was acidified with 10% citric acid and then extracted with EtOAc (2 × 25 mL). The combined EtOAc extracts were dried (Na_2SO_4) and then stripped of EtOAc in vacuo to yield 0.87 g (96%) of **6a** as a white solid. Recrystallization of this material from a mixture of acetone and petroleum ether (bp 30–60 °C) provided an analytical sample: mp 142–143 °C; $[\alpha]^{20}_D$ 5.2° (*c* 1.0, MeOH); NMR ($CDCl_3$) δ 5.2–5.8 (br, 2 H, CO_2H and OH), 4.35 (br d, 1 H, NH), 3.6–4.1 (m, 2 H, NCH and CHO), 2.46 (d, 2 H, CH_2CO), 1.12–1.7 [m with singlet at δ 1.42, 14 H, $CHCH_2$, CH_2, $(CH_3)_3$], 0.9 [d, 6 H, $(CH_3)_2$]. Anal. ($C_{14}H_{27}NO_5$) C, H, N.

5(*S*)-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-hydroxy-7-methyloctanoic Acid (6b). Hydroxy amino acid ester **5b** (1.5 g, 4.7 mmol) was treated with 3 mL of 10% NaOH in a manner

analogous to that described above from **6a**. A yield of 1.4 g (100%) of **6b** was obtained. Recrystallization from a mixture of acetone–petroleum ether (bp 30–60 °C) yielded an analytical sample of **6b**: mp 106–107.5 °C; $[\alpha]^{20}_D$ −12.6° (*c* 1.03, MeOH); NMR ($CDCl_3$) δ 5.04–5.50 (br, 3 H, NH, CO_2H, and OH), 3.92–4.14 (m, 1 H, NCH), 3.50–3.84 (m, 1 H, CHO), 2.44–2.64 (m, 2 H, CH_2CO_2), 1.1–1.76 [m with singlet at δ 1.44, 14 H, $CHCH_2$, CH_2, $(CH_3)_3$], 0.9 [d, 6 H, $(CH_3)_2$]. Anal. ($C_{14}H_{27}NO_5$) C, H, N.

(*R*)-α-Methoxy-α-(trifluoromethyl)-α-phenylacetyl (MTPA) Derivative of Methyl 5(*S*)-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-hydroxy-7-methyloctanoate (7a). The protected hydroxy amino acid **6a** (100 mg, 0.35 mmol) was dissolved in Et_2O and treated with an ethereal solution of diazomethane. The solution was stirred at room temperature for 2 h, after which time the solution was stripped of solvent in vacuo. The residue was redissolved in Et_2O, and this solution was washed with 1 M $NaHCO_3$, followed by saturated NaCl solution. The Et_2O extract was dried ($MgSO_4$) and then stripped of solvent in vacuo. The residue obtained was recrystallized from a mixture of petroleum ether (bp 30–60 °C) and Et_2O to give 80 mg (75%) of the methyl ester of **6a** as fine needles: mp 113–114 °C; $[\alpha]^{20}_D$ +4.4° (*c* 0.5, CH_3OH).

The methyl ester (30 mg, 0.1 mmol) was acylated with (*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetyl chloride by the procedure described by Dale and Mosher.[15] The MTPA derivative of the methyl ester of **6a** was obtained as an oil, which was shown to be homogeneous by TLC: R_f 0.57 in Et_2O–petroleum ether (30–60 °C).

(*R*)-α-Methoxy-α-(trifluoromethyl)-α-phenylacetyl (MTPA) Derivative of Methyl 5(*S*)-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-hydroxy-7-methyloctanoate (7b). The hydroxy amino acid **6b** (100 mg, 0.35 mmol) was converted to its methyl ester by the same procedure described above for the synthesis of the methyl ester of **6a**. The product was obtained as an oil in a yield of 100 mg (94%): $[\alpha]^{25}_D$ −78° (*c* 0.67, CH_3OH). Likewise, this material was converted into its MTPA derivative as described above for **7a**: TLC R_f = 0.57 [Et_2O–petroleum ether (bp 30–60 °C)].

His-Leu-Leu-Val-Phe-OCH_3·2HCl (22). Leu-Leu-Val-Phe-OCH_3·HCl[9] (0.4 g, 0.74 mmol), 1-hydroxybenzotriazole (0.2 g, 1.48 mmol), and N^{α}-(*tert*-butoxycarbonyl)-N^{π}-[(benzyloxy)methyl]-L-histidine (0.28 g, 0.74 mmol) were dissolved in DMF (5 mL). The solution was cooled in an ice bath and then treated with *N*-methylmorpholine (82 mL, 0.74 mmol). A solution of dicyclohexylcarbodiimide (0.15 g, 0.74 mmol) in CH_2Cl_2 was added to the solution. The solution was allowed to warm up to room temperature, where it was allowed to stir overnight. The mixture was cooled in an ice bath, whereupon the precipitate of dicyclohexylurea present was removed by filtration. The solvents were removed in vacuo, and the residue was treated with 50 mL of 1 M $NaHCO_3$. The precipitate that formed was collected and recrystallized from a mixture of H_2O–CH_3OH to give 0.4 g (63%) of N^{α}-Boc-N^{π}-Bom-His-Leu-Leu-Val-Phe-OCH_3: mp 190–193 °C; $[\alpha]^{25}_D$ −50° (*c* 1.0, CH_3OH).

The above product was dissolved in 80% acetic acid (10 mL), and this solution added to a flask containing 10% Pd/C (100 mg). Hydrogen was bubbled into the reaction mixture for 3 h. TLC analysis at this time showed no starting material remaining. The mixture was filtered, and the filtrate was stripped of solvent in vacuo to give 0.31 g (91%) of Boc-His-Leu-Leu-Val-Phe-OCH_3. This material was treated with 10 mL of 4 N HCl in dioxane. The solution was stirred at room temperature for 1.5 h. The dioxane was removed in vacuo, and the residue was dried under vacuum over KOH. Recrystalliation of the residue from a mixture of 1-propanol and Et_2O gave 0.26 g (87%) of product: mp 227–230 °C dec; $[\alpha]^{25}_D$ −25.9° (*c* 0.5, DMF) [lit.[21] mp 226–228 °C dec; $[\alpha]^{25}_D$ −28.4° (*c* 1.0, DMF)].

Synthesis of the Protected and Deprotected Polypeptides Containing (3*R*,5*S*)-AHMOA and (3*S*,5*S*)-AHMOA. The protected and deprotected polypeptides containing (3*R*,5*S*)-AHMOA and (3*S*,5*S*)-AHMOA listed in Tables I and II were synthesized by using the coupling and deprotection procedures described in the synthesis of **22**.

Renin Inhibition Studies. The ability of compounds **16–22** to inhibit either hog kidney renin or human amniotic renin was measured by determining the inhibitory constant (K_i) of each compound. The K_i and the type of inhibition of each compound were determined through the use of Dixon plots.[19] Data for these plots were obtained by measuring the reaction velocities of hog kidney renin or human amniotic renin at two concentrations of porcine angiotensinogen (0.1 and 0.05 μM) in the presence of varying concentrations of each inhibitor.

The enzymatic assay was carried out in a manner identical with that described by us previously.[9,10] Reaction velocities for hog kidney renin were expressed as the number of nanomoles of angiotensin I generated per unit of enzyme per minute, while the reaction velocities for human amniotic renin were expressed as the number of nanomoles of angiotensin I generated per milliliter per hour. The average values of three determinations for each inhibitor concentration at each substrate level were used to generate a Dixon plot ($1/v$ vs. inhibitor concentration) for each compound tested. All lines were calculated by linear regression analysis. The $-[I]$ value at the intersection of the two substrate lines gave the K_i value of each analogue. The competitive and noncompetitive nature of each inhibitor was assessed by whether the point of intersection of the two lines was above or on the *x* axis, respectively.

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